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*Research article/Araştırma makalesi*

# Impact of waterlogging stress on yield components and chemical characteristics of Barley (*Hordeum vulgare*)

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**Abstract**

The aim of this trial was to assess the effect of waterlogging on spike weight, grain weight per spike, dry leave weight, dry culm weight, total weight, contents of chlorophyll and mineral, amino acids and organic acids in barley. Barley under waterlogging stresses exhibited growth reduction and photosynthesis declination as reflected by decline in spike weight grain weight per spike dry leave weight dry culm weight total weight and chlorophyll content. Prolonging waterlogging caused decrease in N, P, K, Ca, Mg, Na, Zn and total amount of minerals; whereas toxic minerals, Fe, Cu and Mn increased. Increased timing in excess water made a considerable increase in levels of amino acids organic acids. While oxalic, propionic, butyric, lactic, citric, malic and abscisic acids increased; decreases were recorded in levels of giberellic, salicylic, indole acetic acids and total organic acids with increasing timing of waterlogging. In conclusion, prolonging waterlogging has significant effect on yield components, levels of minerals, amino acids and organic acids in barley. Ince genotype showed better performance and more resistance to waterlogging than Kalaycı.

**Key words:** Barley, waterlogging, yield components, chemical characteristics

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## Aşırı su stresinin Arpa'da (*Hordeum vulgare*) verim unsurları ve kimyasal bileşenler üzerine etkisi

**Özet**

Bu çalışmada aşırı su basmasının arpada başak ağırlığı, başakta tane ağırlığı, yaprak ve sap kuru ağırlığı, toplam ağırlık, klorofil miktarı, mineral miktarı, amino ve organik asit düzeyleri üzerindeki etkileri belirlenmiştir. Uzayan su basmasına bağlı olarak arpada gelişim geriliklerine ve fotosentez oranında önemli düşüşler belirlenmiş; bunun göstergesi olarak ta başak ağırlığı, başakta tane ağırlığı, yaprak ve sap kuru ağırlığı, toplam kuru ağırlık ve klorofil miktarında önemli düşüşler belirlenmiştir. Uzayan su basmasına bağlı olarak N, P, K, Ca, Mg, Na, Zn ve toplam mineral miktarında önemli düşüşler kaydedilirken; Fe, Cu ve Mn miktarında artışlar kaydedilmiştir. Bu üç elementin (Fe, Cu ve Mn) fotosentezde önemli görev üstlenmelerinin yanısıra aşırı su basmalarında toksit etki yapacak kadar bir artışa neden olduğu belirlenmiştir. Buna ilaveten uzayan su basmasına bağlı olarak amino ve organik asit seviyelerinde artışlar belirlenmiştir. Okzalik asit, propiyonik asit, butirik asit, laktik asit, sitrik asit, malik asit ve absisik asit miktarlarında önemli artışlar belirlenirken gibberelik asit, salisilik asit, IAA ve toplam organik asit düzeylerinde düşüşler kaydedilmiştir. Sonuç olarak uzayan su basmasına bağlı olarak verim unsurları, mineral miktarları, amino asit ve organik asit miktarlarında önemli değişimler ortaya konmakla birlikte; arpa çeşitlerinden İnce arpa genotipi Kalaycı arpa genetipine göre uzayan su basmasına daha dayanıklı arpa çeşiti olduğu belirlenmiştir.

**Anahtar kelimeler:** Arpa, aşırı su basması, verim unsurları, kimyasal içerikler, mineraller, amino ve organik asitler

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## 1. Introduction

Being one of the major crop in cereals, barley (Hordeum vulgare L.) is mostly used for animal feed and malting (Zhou et a., 2007). It is the fourth most important cereal crop after wheat, maize and rice (Setter and Waters, 2003). Barley is grown in many different environments due to adaptability to various environments including irrigated and dry land conditions. Importance of barley has been increasing more and more in this era for not only significant deficit in animal feed and industrial purpose (Jayahar, 2012). Like the other cereals, waterlogging is one of the most stress factors limiting huge amount of barley production in many parts of the world and estimated more than 8 million ha is waterlogged each year (Sayre et al., 1994).

Having two main sources, rainfall and irrigation, water remains on the soil surface for long certain periods without infiltrating the soil. When timing of waterlogging extends, ethanolic fermentation and a number of recovery mechanisms could occur in plants (Setter and Waters, 2003; Pang et al., 2004). Excess water in the root zone by reducing oxygen concentration, creates energy crisis in roots (Colmer and Voesenek, 2009) and reducing plant growth at any growth stage (Setter and Waters, 2003) including yield and yield component (Luxmoore et al., 1973; Gardner and Flood, 1993). As in wheat, barley is very sensitive to waterlogging at sowing time- seedling, flowering, and grain-filling periods; excess water for almost 30 days during these periods reduces plant growth, therefore photosynthesis and grain yield (Luxmoore et al., 1973). Besides, excess water plays important changes in minerals (Setter, 2000), amino acids lit and organic acids (Jayahar, 2012). The aim of this study was to assess the effect of waterlogging on yield components, mineral contents, amino acids and organic acids of barley genotypes.

## 2. Materials and methods

This study, was carried out in greenhouse conditions at Osmangazi University, Agricultural College Eskişehir, Turkey (30°32'E 39°46' N, at an altitude of 792 m) in the 2012–2013 cropping seasons. Seeds were sown in PVC containers (0.75 m width, 1 m length, and 0.75 m height) containing 70 kg of loamy textured soil (31.7% sand, 34.5% silt, and 33.8% clay). Soil also had 0.48% $CaCO_3$, 301.5 mmol/kg $P_2O_5$, 395.1 mmol/kg $K_2O$, and 2.11% organic matter, 6.99 pH, and 2.62 dS/m electrical conductivity. Barley was sown during the first two weeks of September at a seed rate of 475 seed/$m^2$. Sixty kg N $ha^{-1}$ (½ at sowing stage and ½ at tillering stage) and 60 kg $ha^{-1}$ $P_2O_5$ (at sowing) were applied. Ammonium sulfate (21% N) and triple superphosphate (46% $P_2O_5$) were used as fertilizers in the study. Containers in the experiment were protected from bird damage by netting. Two barley genotypes were used: c.v. Kalaycı-97 and Ince-04 are two-rowed, feed barleys. Normal quality water (EC=1.0–2.5 dS $m^{-1}$) was selected in the study. Experimental design was a randomized complete block design (RCBD) with three replications. Normal irrigation as a control (**C**) at sowing, at stem elongation (Feekes 6.0), and at flowering (Feekes 10.51) was applied, and after this stage waterlogging was applied. Barley was allowed to grow until flowering stage and, starting from the beginning of the flowering stage, waterlogging treatments consisted of six treatments: control, 7 days waterlogging (**$W_7$**), 14 days waterlogging (**$W_{14}$**), 21 days waterlogging (**$W_{21}$**), 28 day waterlogging (**$W_{28}$**). Waterlogging was accomplished by using water from a nearby water service, flooding the containers assigned to the waterlogging treatment. Soil was kept saturated with water above field capacity by continuous flooding, usually every day to create an oxygen-deficiency environment.

**Yield component analysis**

Yield components, spike weight (Bhuiya and Kamal, 1994), grain weight per spike (Fathi and Rezaeimoghddam, 2000), dry leave weight (Fathi and Rezaeimoghddam, 2000), dry culm weight (Paull et al., 1988; Kumar and Ramesh, 2001), total weight (Kumar and Ramesh, 2001), chlorophyll content (Uddling et al., 2007) were measured.

**Amino acid analysis**

For the amino acid analysis, 5 mL of 0.1 N HCl was added to 5 mg plant sample. The samples were homogenized and dispersed using an IKA Ultra Turrax D125 Basic homogenizer and incubated at 40°C for 12 hours. Then, the homogenized samples were vortexed. After these sample suspensions were centrifuged at 1200 rpm for 50 minutes, the supernatants were filtered using a 0.22 µm Millex Millipore filter. Next, the supernatants were transferred to vials for amino acid analysis using HPLC as described (Henderson et al. 1999). The quantities of amino acids found in the plant samples, including aspartate, glutamate, and asparagine, were determined after 26 minutes of HPLC derivation and are reported as pmol $\mu l^{-}$

**Organic acid analysis**

For the analysis of organic acids, 10 mL of deionized water was added to mg plant sample, which were homogenized using an IKA Ultra Turrax D125 Basic homogenizer. After centrifugation at 1200 rpm for 50 minutes, the supernatants were filtered through a 0.22 µm pore Millex Millipore filter and collected in vials. The supernatants were subjected to HPLC analysis using a Zorbax Eclipse-AAA 4.6 x 250 mm, 5 µm column (Agilent 1200 HPLC), and the absorbance at 220 nm was read using a UV detector. The flow speed was 1 mL $\mu l^{-1}$, and the column temperature was 250°C. The organic acid contents of the bacterial suspensions, including oxalic and propionic acids, were determined using 25 mM potassium phosphate pH 2.5 as the mobile phase.

**Hormone analysis**

The extraction and purification processes were executed as described (Davies, 1995). For hormone analysis, 5 mL of cold (-40 °C) 80% methanol was added to 5 mg plant sample. The plant suspensions were homogenized for 10 minutes using an IKA Ultra Turrax D125 Basic homogenizer, and then the plant suspensions were incubated for 24 hours in the dark. The plant suspensions were filtered using a Whatman No: 1 filter, and the supernatants were filtered again using a 0.45 µm pore filter. The hormones were analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC), and the absorbance was read at 265 nm using a UV detector. Gibberellic acid, salicylic acid, indole acetic acid (IAA), and abscisic acid (ABA) were determined using 13% acetonitrile (pH 4.98) as the mobile phase.

**Enzyme activities of PGPR**

Phosphatase activity was determined using para-nitro-phenyl phosphate (pNPP) as an ortho-phosphate monoester analog substrate (Tabatabai, 1982). The p-nitrophenol content was determined using a calibration curve obtained with standards containing 0, 10, 20, 30, 40 and 50 ppm of p-nitrophenol.

**Antioxidant enzymes analysis of PGPR**

For antioxidant enzyme assays, frozen plant samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer, pH 7.8, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzyme activities in the apoplastic fractions were measured using a spectrophotometer (Sairam and Srivastava, 2002).

**Element analysis**

The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine the total N content (Bremner, 1996) of PGPR strains. The Ca, Mg, Na, K, P, S, Fe, Cu, Mn, Zn, Pb, Ni and Cd contents were determined using an Inductively Coupled Plasma spectrometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA (Mertens, 2005)

**Statistical analysis**

Data were analysed by SAS and Minitab 15 statistical software programs. Data in yield components sorted by plant species and waterlogging applications differences were identified using the Duncan test option in the analysis of variance (Düzgüneş et al., 1987). Cluster analyses were made to determine similarities/dissimilarities in parameters and waterlowwing applications.

## 3. Results and discussion

Yield and yield components are the effected by crop growth environment, management practices, diseases and pests and formed as a result of genotype x environment interaction in crops (Kumar and Ramesh, 2001). Waterlogging tolerance is termed as resistance to it and sustainability of performance in dry matter production and transportation, relatively yield and yield components for stress conditions such as waterlogging (Setter and Waters, 2003). Depending upon intensity and duration of waterlogging in flowering stage, developmental processes could be defective with inappropriate consequences for both vegetative and generative developmental processes. Exposing barley to excess water during flowering may have a damaging effect on plant grain development, leading to lower yield components (Evans and Wardlaw, 1976). In response to waterlogging significant reduction in grain weight per spike, dry plant weight and chlorophyll occur (Uddling et al., 2007). Similar to their findings significant differences ($p<0.05/0.01$) between waterlogging stress and genotypes and interactions in all yield components except grain weight per spike and chlorophyll content. Significant differences occurred in both genotypes and waterlogging treatments. Kalaycı genotype showed better performance than Ince genotype in spike weight and total weight, whereas Ince genotype had superior capacity in dry leave and culm weights. Yield components decreased with increasing excess water stress and exponential relationship were determined between waterlogging and all yield components (Table 1 and Figure 1). 0.024 g decline in spike weight occurs per day under waterlogging ($y = 1.2498e^{-0,03x}$ $R^2= 0.987$). Decline in $\mathbf{W_7}$ was 1.043 g (12.5%), it was 0.530 g (55.6%) in $\mathbf{W_{28}}$ and by regression it could be reach minimum level with 0.02 g in 49$^{th}$ day. In grain weight per spike, daily declining was 0.023 g ($y = 0.9613e^{-0,046x}$ $R^2= 0.978$). $\mathbf{W_7}$ had 0.752 g (15,5%) loss, $\mathbf{W_{28}}$ had 0.262 g (70.5%) and minimum level was found as 0.007 g in 38$^{th}$ day. Decline in dry leave weight, dry culm weight and total weight per day for waterlogging were 0.101 g ($y = 4.1037e^{-0,076x}$ $R^2= 0.906$), 0.358 g ($y = 14.195e^{-0,047x}$ $R^2= 0.989$) and 0.459 g, ($y = 18.041e^{-0,051x}$ $R^2= 0.981$), respectively. Similarly, minimum levels of dry leave weight, dry culm weight and total weight were found as 0.03 g in 31$^{st}$ day, 0.132 g in 36$^{th}$ day, 0.269 g in 29$^{th}$ day, respectively. $\mathbf{W_7}$ had 2.565 g (16,0% loss), 10.167 g (25.8% loss) and 12.727 g (24.1 loss); while $\mathbf{W_{28}}$ had 0.382 g (87.4% loss), 3.748 g (72.6% loss) and 4.133g (73.3% loss) in dry leave weight, dry culm weight and total weight occurred, respectively. Decline in chlorophyll content per day under waterlogging was found as 1.808 SPAD ($y= 81.841e^{-0,082x}$ $R^2= 0.811$). Decline in $\mathbf{W_7}$ was 49.250 SPAD (7.4%), it was 4.783 SPAD (91.6%) in $\mathbf{W_{28}}$ and by regression it could be reach minimum level with 0.374 SPAD in 32$^{nd}$ day. Besides, while Kalaycı genotype was better in spike weight, grain weight per spike and chlorophyll content, Ince was better in dry leave weight, dry culm weight and total weight. Prolonging waterlogging stress leads significant reduction of photosynthetic and metabolic activities, transportation of photoassimilates, plant growth and development, sterile florets, lowed kernel weights and finally reduced grain yield

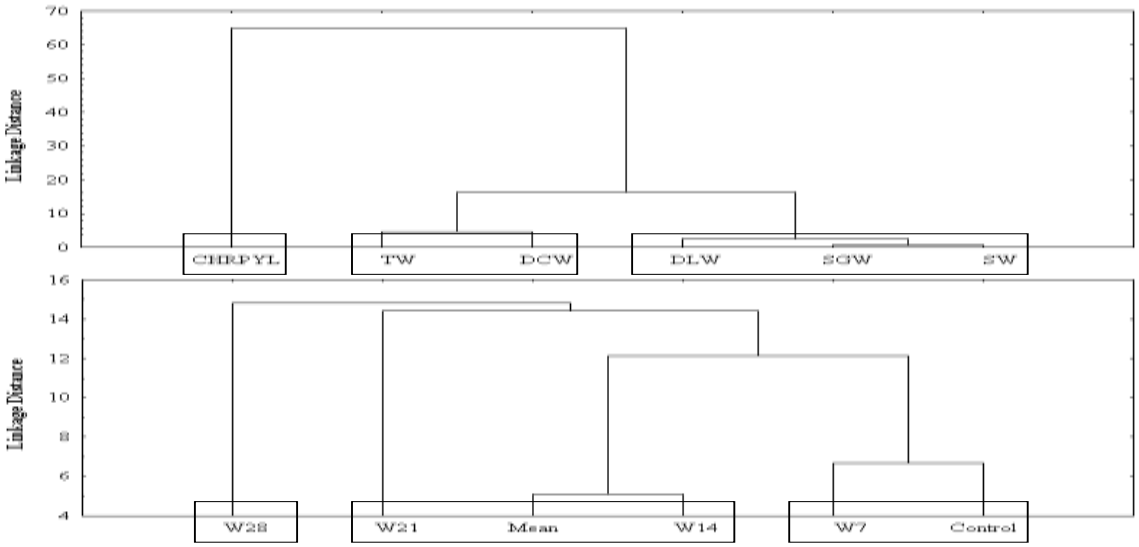
(Uddling et al., 2007). Moreover, cluster analysis showed that three groups occurred in waterlogging stresses and yield components (Figure 1). Total weight (TW), dry culm weight (DCW) created one group, while dry leave weight (DLW), grain weight per spike (SGW) and spike weight (SW) participated in one group. Chlorophyll content (CHRPYL) became alone. Similarly, waterlogging stresses were determined as: **$W_{28}$** alone; **$W_7$** and **Control** in one group; **$W_{21}$**, **$W_{14}$** and **Mean** in the other group (Figure 1).

Table 1. The effect of waterlogging in yield components in barley.

| | Spike Weight (g) | | | Grain Weight per Spike (g) | | | Dry Leave Weight (g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Kalaycı** | **Ince** | **Mean** | **Kalaycı** | **Ince** | **Mean** | **Kalaycı** | **Ince** | **Mean** |
| **C** | 1.270 | 1.117 | 1.193**a** | 0.933 | 0.847 | 0.890**a** | 2.643 | 3.467 | 3.055**a** |
| **$W_7$** | 1.130 | 0.957 | 1.043**b** | 0.773 | 0.730 | 0.752**b** | 1.957 | 3.173 | 2.565**a** |
| **$W_{14}$** | 0.853 | 0.837 | 0.845**c** | 0.543 | 0.447 | 0.495**c** | 1.683 | 2.137 | 1.928**b** |
| **$W_{21}$** | 0.683 | 0.657 | 0.670**d** | 0.347 | 0.330 | 0.338**d** | 1.007 | 0.650 | 0.828**c** |
| **$W_{28}$** | 0.557 | 0.503 | 0.530**d** | 0.280 | 0.243 | 0.262**d** | 0.297 | 0.467 | 0.382**c** |
| **Mean** | 0.899**a** | 0.814**b** | **0.856** | 0.575 | 0.519 | **0.547** | 1.512**b** | 1.986**a** | **1.752** |
| **L.S.D.(%): WL:** 0.149, **G:** 0.067 | | | | **L.S.D.(%): WL:** 0.116 | | | **L.S.D.(%): WL:** 0.493, **G:**0.322, **WL x G:** 0.720 | | |
| **WL:** 73.018**, **G:** 8.028*, **WL x G:** 1.207ns | | | | **WL:** 120.194**, **G:** 3.718ns, **WL x G:** 0.277ns | | | **WL:** 118.380**, **G:** 21.287**, **WL x G:** 7.056** | | |
| | **Dry Culm Weight (g)** | | | **Total Weight (g)** | | | **Chlorophyll Content (SPAD)** | | |
| | **Kalaycı** | **Ince** | **Mean** | **Kalaycı** | **Ince** | **Mean** | **Kalaycı** | **Ince** | **Mean** |
| **C** | 12.147 | 15.277 | 13.712**a** | 14.793 | 18.737 | 16.765**a** | 53.833 | 52.500 | 53.167**a** |
| **7 Day** | 9.273 | 11.060 | 10.167**b** | 11.227 | 14.227 | 12.727**b** | 49.267 | 49.233 | 49.250**a** |
| **14 Day** | 6.183 | 8.650 | 7.417**c** | 7.863 | 10.827 | 9.345**c** | 38.167 | 37.733 | 37.950**b** |
| **21 Day** | 4.617 | 5.357 | 4.987**cd** | 5.627 | 6.003 | 5.815**d** | 22.333 | 16.533 | 19.433**c** |
| **28 Day** | 3.613 | 3.883 | 3.748**d** | 3.913 | 4.353 | 4.133**d** | 4.833 | 4.733 | 4.783**d** |
| **Mean** | 7.167**b** | 8.845**a** | **8.006** | 8.685**b** | 10.939**a** | **9.757** | 33.687 | 32.147 | **32.917** |
| **L.S.D.(%): WL:** 2.484, **G:**0.875, **WL x G:** 1.377 | | | | **L.S.D.(%): WL:** 2.735, **G:**0.757, **WL x G:** 1.714 | | | **L.S.D.(%): WL:** 5.082 | | |
| **WL:** 59.156**, **G:** 36.934**, **WL x G:** 3.673* | | | | **WL:** 79.246**, **G:** 78.590**, **WL x G:** 9.114** | | | **WL** 365.214**, **G:** 1.463ns, **WL x G:** 0.733ns | | |

***:** significant at 5%, ****:** significant at 1% and **ns:** no significant at 5%; **WL:** Waterlogging, **G:** Genotype

Mineral nutrients, taken from soil, are classified macro and micro nutrients. Macro nutrients are N, P, K, Ca and Mg. N, P, K and Mg are essential for plant growth, various biochemical processes such as photosynthesis and proteins synthesis etc (Baque et al., 2006). Ca is vital for strength of plant structure and element transport (Trought and Drew, 1982). Excess water may make changes in availability of mineral nutrients by either removal or oxidation or leaching (Huang, 2001); nitrogen, phosphorus, potassium and calcium decreases, however iron, sodium and chloride increases, finally mineral nutrient deficiencies and microelement toxicities occur (Hocking et al., 1987; Setter et al., 1997). N deficiency may be induced by the low redox potential in waterlogged soils promoting denitrification of $NO_3$.




**Chrpyl:** Clorophyll content, **T.W:** Total Weight, **D.C.W:** Dry culm weight, **D.L.W:** Dry leave weight, **S.G.W:** Grain weight per spike, **S.W:** Spike weight

Figure 1. Dendogram of yield components and waterlogging applications in barley.

In waterlogged soils, root metabolism and root growth are inhibited, deficiency in $O_2$ plays important role in plant energy status (Drew 1991); besides leaf superoxide dismutase activity, leaf catalase activity, and root oxidiazibility reduced and this phenomenon decrease N, P, K, Mg, Zn and Ca uptake (Zhou et al., 2007). Our findings were similar to other studies that decreases in N, P, K, Zn, Mg, Ca and total mineral uptake were found and exponential relationship were determined between waterlogging and all mineral contents (Table 2). Decreases on N in **$W_{28}$** versus **C**

were observed as 24.32% in Kalaycı genotype and as 11.32% in Ince genotype. Besides, as a mean of genotypes decrease in N 17.59%. Decline in N per day occurred as % 0.011 in waterlogging ($y= 2.004e^{-0,006x}$ $R^2=0.940$) . Moreover, Zn decreased as 11.90% in Kalaycı genotype, whereas Ince barley had 10.73% increase and 0.23% decrease has taken place in mean. 0.01 mg/kg decline in Zn occurs per day under waterlogging ($y= 27.092e^{-3E-04x}$ $R^2=0.118$). In genotypes, Ince in N, P, K, Fe, Cu, Mn, Zn and total; Kalaycı in Mg, Ca and Na showed higher performance.

Table 2. The effect of waterlogging in minerals in barley.

| | | % | mg/kg | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **N** | **P** | **K** | **Ca** | **Mg** | **Na** | **Fe** | **Cu** | **Mn** | **Zn** | **Total** |
| **Kalaycı** | **C** | 1.85 | 2542.66 | 3526.34 | 450.12 | 756.32 | 86.51 | 28.51 | 1.32 | 8.51 | 25.63 | 7426,77 |
| | **$W_7$** | 1.76 | 2456.75 | 3485.56 | 442.34 | 745.24 | 85.27 | 32.15 | 1.65 | 10.12 | 26.51 | 7285,19 |
| | **$W_{14}$** | 1.70 | 2401.12 | 3265.02 | 431.26 | 721.65 | 82.38 | 36.17 | 2.45 | 14.25 | 24.15 | 6978,72 |
| | **$W_{21}$** | 1.62 | 2235.77 | 3102.64 | 426.76 | 702.06 | 80.86 | 38.41 | 3.47 | 20.36 | 23.26 | 6633,12 |
| | **$W_{28}$** | 1.40 | 2035.58 | 2865.45 | 415.87 | 685.36 | 76.37 | 42.45 | 3.4 | 25.63 | 22.58 | 6171,46 |
| **Mean** | | 1,66 | 2334.37 | 3249.00 | 433.27 | 722.12 | 82.27 | 35.53 | 2.45 | 15.77 | 24.42 | 6899.05 |
| **Ince** | **C** | 2.12 | 2815.26 | 3754.11 | 445.13 | 775.51 | 80.38 | 30.15 | 1.45 | 12.14 | 27.31 | 7942,17 |
| | **$W_7$** | 2.03 | 2756.45 | 3688.63 | 432.65 | 712.56 | 76.44 | 34.25 | 1.65 | 15.24 | 29.65 | 7746,82 |
| | **$W_{14}$** | 2.02 | 2745.18 | 3541.48 | 410.24 | 703.58 | 75.35 | 38.69 | 2.45 | 19.85 | 28.54 | 7565,55 |
| | **$W_{21}$** | 1.95 | 2602.66 | 3421.37 | 400.08 | 685.43 | 70.53 | 41.47 | 3.67 | 18.62 | 32.15 | 7285,86 |
| | **$W_{28}$** | 1.88 | 2458.42 | 3269.88 | 368.43 | 680.75 | 62.12 | 49.65 | 3.98 | 24.51 | 30.24 | 6947,26 |
| **Mean** | | 2,00 | 2675.59 | 3535.09 | 411.31 | 711.57 | 72.96 | 38.84 | 2.64 | 18.07 | 29.58 | 7497.53 |
| **Means of Genotypes** | **C** | 1.99 | 2678.96 | 3640.23 | 447.63 | 765.92 | 83.45 | 29.33 | 1.39 | 10.33 | 26.47 | 7684,47 |
| | **$W_7$** | 1.90 | 2606.60 | 3587.10 | 437.50 | 728.90 | 80.86 | 33.20 | 1.65 | 12.68 | 28.08 | 7516,01 |
| | **$W_{14}$** | 1.86 | 2573.15 | 3403.25 | 420.75 | 712.62 | 78.87 | 37.43 | 2.45 | 17.05 | 26.35 | 7272,14 |
| | **$W_{21}$** | 1.79 | 2419.22 | 3262.01 | 413.42 | 693.75 | 75.70 | 39.94 | 3.57 | 19.49 | 27.71 | 6959,49 |
| | **$W_{28}$** | 1.64 | 2247.00 | 3067.67 | 392.15 | 683.06 | 69.25 | 46.05 | 3.69 | 25.07 | 26.41 | 6559,36 |
| **Mean** | | 1,84 | 2504.99 | 3392.05 | 422.29 | 716.85 | 77.63 | 37.19 | 2.55 | 16.92 | 27.00 | 7198.29 |

Decreases in **$W_{28}$** reached to 19.94% in Kalaycı genotype, 12,68% in Ince genotype and 16.12% in mean for P. Daily P decrease was found as 15.01 mg/kg ($y= 2723.5e^{-0,006x}$ $R^2=0.917$). In the same way, similar trends occurred in K, Ca and Mg. Should be rewritten 18.74%, 12.90% and 15.73% in K, 7.61%, 17.23% and 12.39% in Ca, and 9.38%, 12.22% and 10.82% in Mg. Decreases per day in K, Ca and Mg were 21.00 mg/kg ($y= 3696e^{-0,006x}$ $R^2=0.967$), 1.92 mg/kg ($y= 449.92e^{-0,005x}$ $R^2=0.967$) and 2.87 mg/kg ($y= 757.38e^{-0,004x}$ $R^2=0.957$), respectively. On the other hand, changes in available ion concentration, electron excess, reductions from $Fe^{+3}$ and $Mn^{+4}$ to $Fe^{+2}$ and $Mn^{+2}$ occur. Barley are not capable of oxidize Fe and Mn and Mn and Fe reached toxic level under waterlogged conditions (Mengel and Kirkby, 2001). Waterlogging decreased oxygen diffusion and reduced ion uptake, such as N, P, K., Ca, Mg and Zn, and caused to increase in Na, Fe, Cu and Mn (Najafi et al., 2012). In our study, Na decreased while increased waterlogging. Na in Kalaycı genotype, Ince genotype and mean were decreased by 11.72%, 22.72% and 17.02%, respectively (Table 2). Daily decline in Na occurs as 0.48 mg/kg under waterlogging ($y= 84.605e^{-0,006x}$ $R^2=0.926$). Significant increases were found in Kalaycı genotype, Ince genotype and mean for Fe, Cu and Mn (48.90%, 64.68% and 57.01% in Fe, 157.58%, 174.48% and 165.47% in Cu, 201.18%, 101.89% and 142.69% in Mn). Increase per day in Fe, Cu and Mn were determined as 0.57 mg/kg ($y= 29.628e^{0,0155x}$ $R^2=0.989$), 0.09 mg/kg ($y= 1.3889e^{0,0389x}$ $R^2=0.945$) and 0.52 mg/kg ($y= 10.464e^{0,0315x}$ $R^2=0.987$), respectively.

Meanwhile, Figure 2 showed that both waterlogging applications and minerals formed three groups. **$W_{28}$** seemed alone, while **$W_7$** and **C**; **$W_{14}$, $W_{21}$** and mean formed in two and three groups, respectively. K and P, Mg and Ca were grouped two; Na, Zn, Mn, Fe, Ca and N created one group. We can conclude that increasing waterlogging leads rapid reductions in most minerals such as N, P, K., Ca, Mg except Fe, Cu, Mn that are toxic minerals (Table 2). Moreover, amino acids are essential components of metabolic activities and proteins and these are drastically required to draw up crop yield and quality in cereals (Ashraf et al., 2013). Not only yield and yield components but amino acids are significantly affected from stress conditions.

Amino acids are building parts of crops, and they play vital role on protein synthesis, photosynthesis, action of stomas, chelating effect, pollination and fruit formation and stress resistance. Amino Acids make a assistance crop to prevent or recuperate against flooding (Frizzi et al., 2008). Table 3 shows changes of amino acids with prolonging waterlogging. Ince genotype had higher amino acid levels in all amino acids. Increased time of waterlogging causes

increase in accumulation of amino acids (Ashraf et al., 2013). Asparagine, β-alanine, cysteine, cystine, isoleucine, lysine, γ-methylene glutamine, phenylalanine, proline, tyrosine and tryptophan in the seed and asparagine, cysteine, phenylalanine, tryptophan and valine significantly increased by prolonging waterlogging (Asha and Rao, 2002).

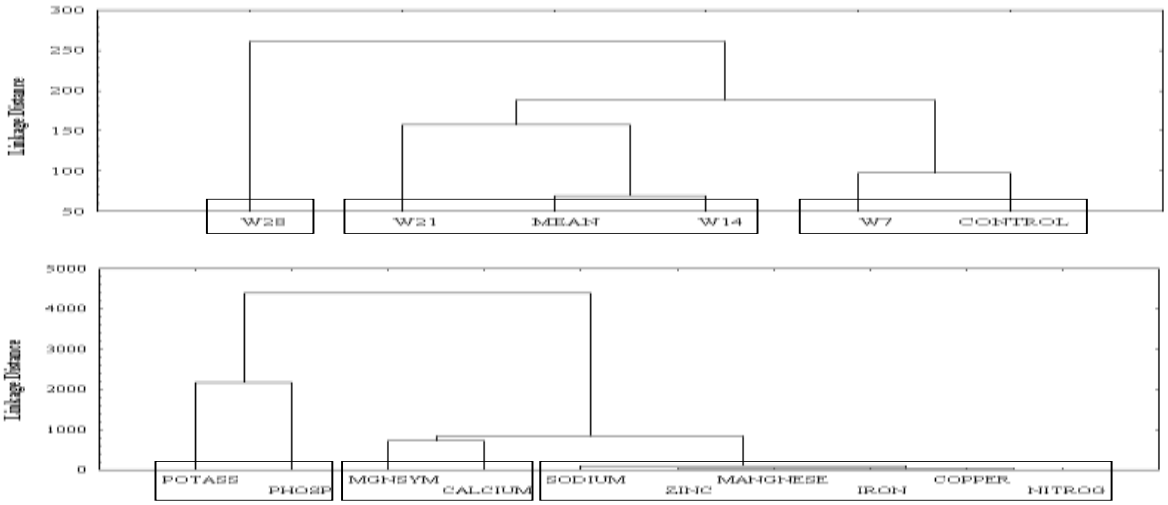


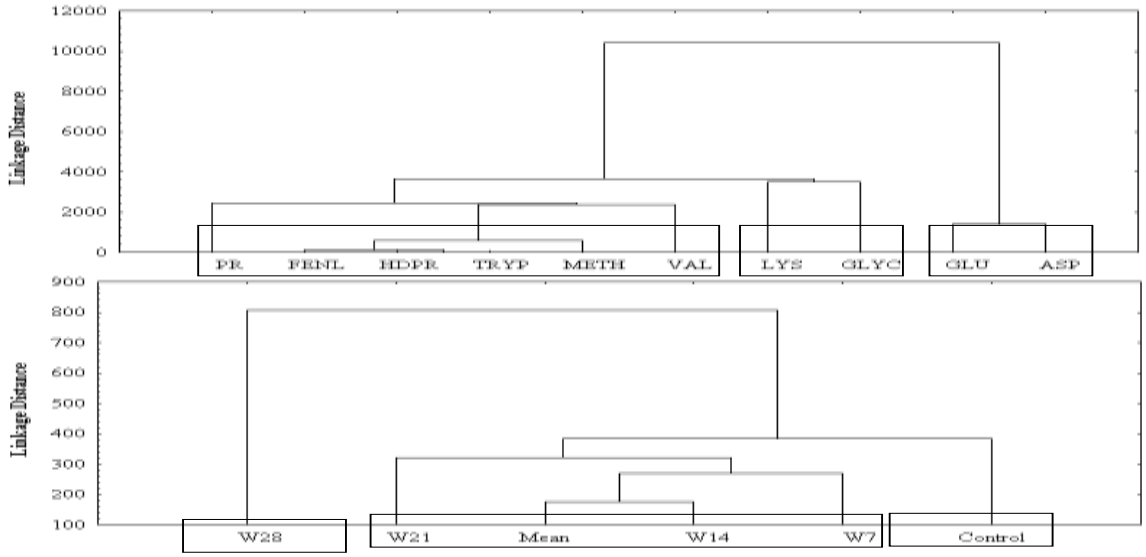

Figure 2. Dendogram of minerals and waterlogging applications in barley.

Table 3. The effect of waterlogging in amino acids in barley.

| | pmol/ul | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Asparagine** | **Glutamine** | **Glycine** | **Valine** | **Methionine** | **Tryptophan** | **Phenylalanine** | **Lysine** | **Hydroxy Proline** | **Proline** | **Total Amino Acids** |
| | | | | | | **Kalaycı** | | | | | |
| **C** | 8914.23 | 8265.98 | 3309.64 | 1011.36 | 2098.76 | 1811.68 | 1799.47 | 4751.86 | 1785.46 | 112.97 | 33861.41 |
| **$W_7$** | 9260.16 | 8770.56 | 3563.52 | 1040.64 | 2192.64 | 1968.00 | 1925.76 | 4878.72 | 1969.92 | 125.26 | 35695.18 |
| **$W_{14}$** | 9404.85 | 8907.60 | 3619.20 | 1056.90 | 2226.90 | 1998.75 | 1955.85 | 4954.95 | 2000.70 | 127.22 | 36252.92 |
| **$W_{21}$** | 9790.69 | 9273.04 | 3767.68 | 1100.26 | 2318.26 | 2080.75 | 2036.09 | 5158.23 | 2082.78 | 132.44 | 37740.22 |
| **$W_{28}$** | 10224.76 | 9684.16 | 3934.72 | 1149.04 | 2421.04 | 2173.00 | 2126.36 | 5386.92 | 2175.12 | 138.31 | 39413.43 |
| **Mean** | 9520.60 | 9017.23 | 3663.74 | 1069.91 | 2254.31 | 2023.35 | 1979.92 | 5015.93 | 2025.32 | 128.78 | 36699.11 |
| | | | | | | **Ince** | | | | | |
| **C** | 10121.00 | 9452.00 | 3755.00 | 1149.04 | 2376.00 | 2054.00 | 2025.00 | 5247.00 | 2086.00 | 126.00 | 38391.04 |
| **$W_7$** | 10431.80 | 9821.20 | 3990.40 | 1165.30 | 2455.30 | 2203.75 | 2156.45 | 5463.15 | 2205.90 | 140.27 | 40033.52 |
| **$W_{14}$** | 10625.88 | 10003.92 | 4064.64 | 1186.98 | 2500.98 | 2244.75 | 2196.57 | 5564.79 | 2246.94 | 142.88 | 40778.33 |
| **$W_{21}$** | 10868.48 | 10232.32 | 4157.44 | 1214.08 | 2558.08 | 2296.00 | 2246.72 | 5691.84 | 2298.24 | 146.14 | 41709.34 |
| **$W_{28}$** | 11450.72 | 10780.48 | 4380.16 | 1279.12 | 2695.12 | 2419.00 | 2367.08 | 5996.76 | 2421.36 | 153.97 | 43943.77 |
| **Mean** | 10699.58 | 10057.98 | 4069.53 | 1198.90 | 2517.10 | 2243.50 | 2198.36 | 5592.71 | 2251.69 | 141.85 | 40971.20 |
| | | | | | | **Means of Genotypes** | | | | | |
| **C** | 9521.78 | 8951.40 | 3594.30 | 1075.87 | 2244.35 | 1975.13 | 1940.28 | 4973.93 | 1992.05 | 123.35 | 36392.42 |
| **$W_7$** | 9845.98 | 9295.88 | 3776.96 | 1102.97 | 2323.97 | 2085.88 | 2041.11 | 5170.94 | 2087.91 | 132.76 | 37864.35 |
| **$W_{14}$** | 10015.37 | 9455.76 | 3841.92 | 1121.94 | 2363.94 | 2121.75 | 2076.21 | 5259.87 | 2123.82 | 135.05 | 38515.62 |
| **$W_{21}$** | 10329.59 | 9752.68 | 3962.56 | 1157.17 | 2438.17 | 2188.38 | 2141.41 | 5425.04 | 2190.51 | 139.29 | 39724.78 |
| **$W_{28}$** | 10837.74 | 10232.32 | 4157.44 | 1214.08 | 2558.08 | 2296.00 | 2246.72 | 5691.84 | 2298.24 | 146.14 | 41678.60 |
| **Mean** | 10110,09 | 9537,61 | 3866,64 | 1134,41 | 2385,70 | 2133,43 | 2089,14 | 5304,32 | 2138,51 | 135,32 | 38835,15 |

All amino acid levels increased with prolonging waterlogging and these increased were determined as exponential. Increases as a percentage on all amino acids in Kalaycı and Ince barley genotypes were 13.36-22.43% and 11.32-22.20%, respectively, besides mean increase occurred as 13.87-22.31%. It was interesting that similar increases occurred in amino acids rates. Increases were higher in Kalaycı genotypes than the other one. Increases as mg/kg in

prolonging waterlogging (**$W_{28}$** versus **C**) for asparagine, glutamine and glycine in Kalaycı genotype, Ince genotype and mean were 1310.53, 1329.73 and 1320.13; 1418.18, 1328.48 and 1373.33; 625.08, 625.16 and 625.16, respectively. Meanwhile, these increases (as mg/kg) in valine, methionine, tryptophan, fenilananine and lysine in Kalaycı genotype, Ince genotype and mean were found as 137.68, 130.08 and 133.88; 322.28, 319.12 and 320.70; 361.32, 365.00 and 363.16; 326.89, 342.08 and 334.49; and 635.06, 749.76 and 692.41, respectively (Table 3). Proline as an anti-stress organic molecule is another amino acid that accumulates so fast in crop and it is well indicator when crop induces stress condition. Moreover, its conversion to hydroxyl proline takes place from proline residues in polypeptide chain (Capone et al., 2004). Hydroxy proline and proline increased with prolonged waterlogging (increase of hydroxy proline and proline in Kalaycı genotype, Ince genotype and mean; 389.66, 335.36 and 362.51; and 25.34, 27.97 and 26.65, respectively). Asha and Rao (2002) reported that amino acid synthesis (asparagine, β-alanine, cysteine, cystine, isoleucine, lysine, γ-methylene glutamine, phenylalanine, proline, tyrosine and tryptophan) increased after 96 h of waterlogging and fresh synthesis and accumulation of amino acids occurred with increased time of waterlogging.




**Pr:** Proline, **Fenl:** Fenilalanine, **Hdpr:** Hydroxy proline, **Tryp:** Tryptophan, **Meth:** Methionine, **Val:** Valine, **Lys:** Lysine, **Glyc:** Glycine, **Glu:** Glutamine, **Asp:** Asparagine

Figure 3. Dendogram of amino acids and waterlogging applications in barley.

Daily increases in amino acids versus waterlogging as a mg/kg were 44.78 ($y= 9500.6e^{0,0044x}$ $R^2=0.974$) in asparagine, 39.80 in glutamine ($y= 8946.8e^{0,0045x}$ $R^2=0.976$), 16.17 in glycine ($y= 3609.6e^{0,0048x}$ $R^2=0.977$), 4.72 in valine ($y= 1069.8e^{0,0041x}$ $R^2=0.963$), 9.95 in methionine ($y= 2240.6e^{0,0044x}$ $R^2=0.975$), 8.93 in tryptophan ($y= 1987.5e^{0,005x}$ $R^2=0.973$), 8.74 in phenylalanine ($y= 1949.4e^{0,0049x}$ $R^2=0.976$), 22.14 in lysine ($y= 4973.6e^{0,0045x}$ $R^2=0.977$), 8.94 in hydroxy proline ($y= 1998.4e^{0,0048x}$ $R^2=0.977$), 0.56 in proline ($y= 125.07e^{0,0055x}$ $R^2=0.955$) (Table 3). Gadallah (1994) reported that having lower osmotic potential due to solutes accumulation and high root resistance to water flow plants under waterlogging has reduced soluble proteins and increased free amino acid levels. Increased synthesis and decreased utilization of amino acids or inhibition of them for protein synthesis under waterlogging caused increase in free amino acids. Dendogram in Figure 3 shows grouping of amino acids and timings waterlogging. Both amino acids and timings waterlogging were grouped in three. When **$W_{28}$** and **C** draw alone groups; **$W_7$**, **$W_{14}$**, **$W_{21}$** and mean participated in same group. On the other glutamine and asparagine, lysine and glycine were grouped into twos. Proline, valine, methionine, tryptophan, phenylalanine and hydroxy proline created one group.

Crops seem to have varieties of organic acids that bear vital role in anabolic and catabolic reactions comprising of energy production, making up leading indicators amino acid synthesis and regulating crop to environmental conditions (Gleixner and Mügler, 2007). Besides, produced mainly in mitochondria and stored in vacuole, organic acids act a part in photosynthesis, nutrient deficiencies, metal tolerance and stress conditions (Roberts et al., 1984). A number of studies reported that crops are significantly damaged from soil waterlogging by causing reduce in metabolites such as organic acids. Besides, in hypoxia or anoxia the pyruvic acid is partially oxidized to in some chemical compounds including ethanol, aldehydes, and organic acids (oxalic, lactic, butyric, citric acids etc.) that harm plant metabolism in case of high levels (Setter and Waters, 2003). $O_2$ shortage caused by waterlogging restricts respiration , electron transport and ATP formation; unbalance of oxidation and reduction stability between cell membranes and increases membrane permeability. This causes increases in level of solutes inducing increase in amount of carbohydrates, amino acids, organic acids, ions and electric conductivity (Blum and Ebercon, 1981; Al-Ani et al., 2011). Besides, function and level of organic acids in plants are rigorously related to other biochemical activities in plants and relationship between plant-soil conditions (Johnson et al., 1994). In the study, relationship between waterlogging and all organic acid levels were found as exponential. Prolonging waterlogging caused increase in oxalic

propionic butyric and lactic acids level. Accumulation of oxalic, propionic, malic, citric and butyric acids could be from these consequences. Increases (**$W_{28}$** versus **C**) as % in Kalaycı genotype, Ince genotype and mean for oxalic propionic, butyric and lactic acids were as 15.45%, 12.96% and 14.11%; 28.66%, 14.74% and 20.93%; 13.07%, 12.99% and 13.02%, respectively. Moreover, increases on Kalaycı genotype, Ince genotype and mean in citric and malic acids were as 18.08%, 11.27% and 14.50%; and 19.25%, 13.06% and 15.91%, respectively. Increases per day waterlogging in oxalic, propionic, butyric, lactic, citric and malic acids as ng/ul were 0.005 ($y= 1.0954e^{0,0042x}$ $R^2=0.978$), 0.007 ($y= 1.7384e^{0,0041x}$ $R^2=0.960$), 0.002 ($y= 3.8493e^{0,0041x}$ $R^2=0.960$), 0.08 ($y= 16.797e^{0,0041x}$ $R^2=0.964$), 0.001 ($y= 2.4854e^{0,0041x}$ $R^2=0.967$) and 0.006 ($y= 1.492e^{0,004x}$ $R^2=0.953$), respectively (Table 4). Ince genotype had higher organic acid levels in all organic acids. Giberellic, indol acetic acid and salicylic acid act in plant growth and development, photosynthesis, transpiration, ion uptake and transport, elongation and division of cells Negative environmental effects such as waterlogging in level of such organic acids (Fuchs and Leberman, M., 1968).

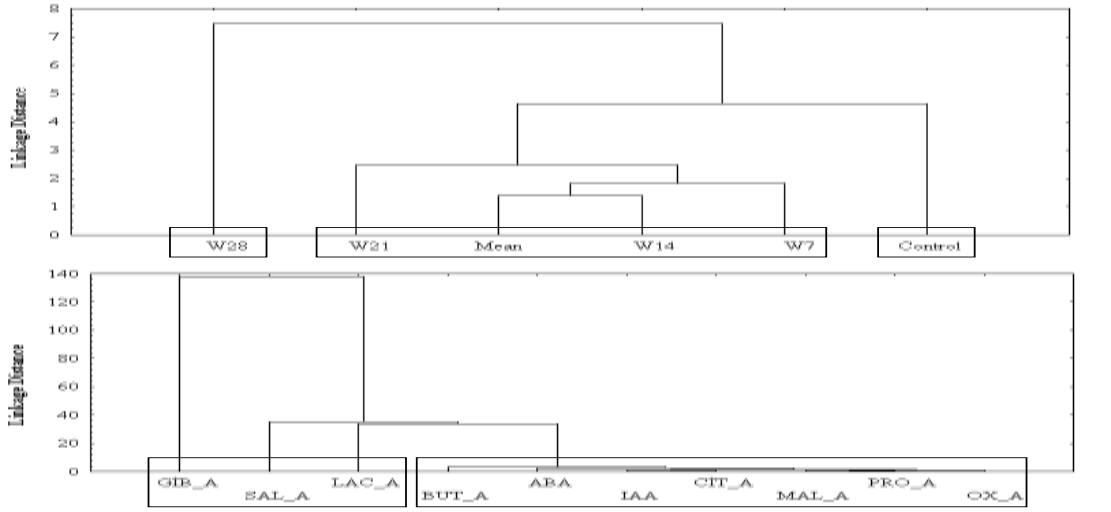
Table 4. The effect of waterlogging in organic acids in barley.

ng/ul

| | Oxalic Acid | Propionic Acid | Butyric Acid | Lactic Acid | Citric Acid | Malic Acid | Giberellic Acid | Salicylic Acid | Indole Acetic Acid | Abscisic Acid | Total Organic Acids |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kalaycı** | | | | | | | | | | | |
| **C** | 1.02 | 1.63 | 3.61 | 15.74 | 2.33 | 1.40 | 89.08 | 32.23 | 2.39 | 0.13 | 149.55 |
| **$W_7$** | 1.06 | 1.69 | 3.74 | 16.34 | 2.42 | 1.45 | 84.74 | 30.66 | 2.27 | 0.15 | 144.52 |
| **$W_{14}$** | 1.07 | 1.72 | 3.80 | 16.59 | 2.46 | 1.47 | 81.86 | 29.61 | 2.19 | 0.16 | 140.94 |
| **$W_{21}$** | 1.12 | 1.79 | 3.96 | 17.28 | 2.56 | 1.53 | 78.00 | 28.22 | 2.09 | 0.18 | 136.72 |
| **$W_{28}$** | 1.17 | 1.87 | 4.13 | 18.04 | 2.67 | 1.60 | 67.41 | 24.39 | 1.81 | 0.24 | 123.32 |
| **Mean** | 1.09 | 1.74 | 3.85 | 16.80 | 2.49 | 1.49 | 80.22 | 29.02 | 2.15 | 0.17 | 139.01 |
| **Ince** | | | | | | | | | | | |
| **C** | 1.19 | 1.87 | 4.13 | 18.04 | 2.67 | 1.60 | 102.08 | 36.93 | 2.73 | 0.15 | 171.39 |
| **$W_7$** | 1.20 | 1.89 | 4.19 | 18.30 | 2.71 | 1.63 | 97.74 | 35.36 | 2.62 | 0.18 | 165.83 |
| **$W_{14}$** | 1.23 | 1.93 | 4.27 | 18.64 | 2.76 | 1.66 | 97.26 | 35.19 | 2.61 | 0.22 | 165.75 |
| **$W_{21}$** | 1.25 | 1.97 | 4.37 | 19.06 | 2.82 | 1.69 | 93.89 | 33.97 | 2.52 | 0.23 | 161.78 |
| **$W_{28}$** | 1.32 | 2.08 | 4.60 | 20.08 | 2.97 | 1.78 | 90.52 | 32.75 | 2.43 | 0.26 | 158.80 |
| **Mean** | 1.24 | 1.95 | 4.31 | 18.82 | 2.79 | 1.67 | 96.30 | 34.84 | 2.58 | 0.21 | 164.71 |
| **Means of Genotypes** | | | | | | | | | | | |
| **C** | 1.10 | 1.75 | 3.87 | 16.89 | 2.50 | 1.50 | 95.58 | 34.58 | 2.56 | 0.14 | 160.47 |
| **$W_7$** | 1.13 | 1.79 | 3.97 | 17.32 | 2.56 | 1.54 | 91.24 | 33.01 | 2.44 | 0.17 | 155.17 |
| **$W_{14}$** | 1.15 | 1.82 | 4.04 | 17.62 | 2.61 | 1.56 | 89.56 | 32.40 | 2.40 | 0.19 | 153.35 |
| **$W_{21}$** | 1.19 | 1.88 | 4.16 | 18.17 | 2.69 | 1.61 | 85.95 | 31.09 | 2.30 | 0.21 | 149.25 |
| **$W_{28}$** | 1.24 | 1.97 | 4.37 | 19.06 | 2.82 | 1.69 | 78.97 | 28.57 | 2.12 | 0.25 | 141.06 |
| **Mean** | 1.16 | 1.84 | 4.08 | 17.81 | 2.64 | 1.58 | 88.26 | 31.93 | 2.36 | 0.19 | 151.86 |

Similar to this, prolonging excess water resulted in retardation, deterioration in plant growth, metabolic activities and decline level of giberellic, Indol acetic acid and salicylic acids in the study. Abscisic acid has vital importance to cope with environmental stress and adaptation of ambient conditions. When crop induce excess water stress, therefore $O_2$ shortage; abscisic acid is produced, level of it increases with regulating some biochemical and physiological processes such as leaf water potential, stomatal opening, osmoregulation, reductions in growth and productivity (Blum and Ebercon, 1981). Besides, we found that level of total organic acid decreased with increasing effect of waterlogging. Similar to literatures, decreases of giberellic, salicylic, indole acetic acids in Kalaycı genotype, Ince genotype and mean were as 25.27%, 10.85% and 17.64%; 21.96%, 9.63% and 15.34%; 25.06%, 8.14% and 16.21%, respectively. Decreases per day waterlogging in giberellic, salicylic, indole acetic acids as ng/ul were 0.550 ($y= 96.244e^{-0,006x}$ $R^2=0.941$), 0.199 ($y= 34,82e^{-0,006x}$ $R^2=0.941$) and 0.02 ($y= 2,5753e^{-0,006x}$ $R^2=0.943$), respectively (Table 4). Increases on Kalaycı genotype, Ince genotype and mean in abscisic acid were as 118.18%, 52.94% and 78.57%, respectively. Increase per day waterlogging in abscisic acid was 0.004 ng/ul ($y= 0,1437e^{0,0196x}$ $R^2=0.984$). Dendogram of organic acids was given in Figure 3 organic acid groups and timings waterlogging. Similar to amino acid dendogram, **$W_{28}$** and **C** accounted for alone groups; then other ones (**$W_7$, $W_{14}$, $W_{21}$** and mean) comprised same group.

Giberellic, salicylic and lactic acids presented one group, such group included butyric, abscisic, indol acetic, citric, malic, propionic and oxalic acids (Figure 4).




**Gib.A:**Giberellic Acid, **Sal. A:** Salicylic Acid, **Lac. A:** Lactic Acid, **But. A:** Butyric Acid, **ABA:** Absisic Acid, **IAA:** Indole Acetic Acid, **Cit. A:** Citric Acid, **Mal. A:** Maleic Acid, **Pro. A:** Propionic Acid, **Ox. A:** Oxalic Acid.

Figure 4. Dendogram of organic acids and waterlogging applications in barley.

Consequently, prolonging waterlogging has significant effect in barley. Ince genotype showed better performance and more resistance to waterlogging than Kalaycı. Barley under waterlogging stresses exhibited growth reduction and photosynthesis declination by determining significant decline in spike weight grain weight per spike dry leave weight dry culm weight total weight and chlorophyll content. Prolonging waterlogging caused decrease in N, P, K, Ca, Mg, Na, Zn and total amount of minerals; whereas toxic minerals, Fe, Cu and Mn increased. Increased timing in excess water made a considerable changes in amino and organic acid levels. Levels of amino acids, asparagine, glutamine, glycine, valine, methionine, tryptophan, phenylalanine, lysine, hydroxy proline, proline and total amino acids increased with excess water stress. In organic acids, while oxalic, propionic, butyric, lactic, citric, malic and abscisic acids increased; decreases were recorded in levels of giberellic, salicylic, indole acetic acids and total organic acids with increasing timing of waterlogging.

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